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SEASONAL VARIATION IN LEAF EXTRACTS OF *DENDROCALAMUS STRICTUS* SPS-AS REFLECTED IN DIFFERENTIAL EXPRESSION OF SOLUBLE BIOSYNTHATES

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ABSTRACT

A variation within species is the reflection of its genotype and its interaction with the environment. Seasonal cycles of temperature, photoperiod, light intensity, relative humidity and rainfall exert a pronounced control over the various biosynthetic pathways and provide it with a pattern to which developmental changes are physiologically locked. The present study carried out on the germplasm of D.strictus (raised via offsets collected from different eco-geographic zones of India with an altitudinal sprawl of 225-1367 mts) revealed substantial variation in biochemical characteristics in different seasons March –May, June-Aug and Dec-Jan.

Significant inter-accession variation was observed among D.strictus sps. The maximum mean value of chlorophyll content (2.551 mg/gm), total soluble protein (18.02 mg/gm) and soluble sugars (25.24 mg/gm) were observed in A35 from Hoshiarpur,India. The average value of phenol content among the accessions was found to be 0.20 mg/gm. The accession A32 (0.3 mg/gm) from Kahanpur registered maximum mean value for phenol content. Overall significant seasonal differences in biochemical characteristics reflect the influence of extraneous factors underscoring the reciprocal relationship between genotype and environmental factors. The best association between season* genotypes with respect to photosynthates was mostly observed in the months of July-August in accessions from Punjab (A35).

KEYWORDS: D.Strictus, Diversity, Germplasm, Biochemical Variation

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INTRODUCTIONs

Both anthropogenic activities such as over exploitation compounded with gregarious flowering in *D. strictus* have resulted in major depletion of its growing area and diversity, thus there is an urgent need to increase its plantation with high productivity, which can be achieved through ascertaining the potential species, analysis of genetic variability among traits and association of a particular character in relation to other traits contributing to yield (Mary and Goplan, 2006). The population level genetic diversity in *D. strictus* is still at its infancy stage. Efforts to quantitatively assess genetic diversity would assist in proper selection of desired genotype for bamboo improvement programs. Germplasm characterization is an important link between conservation and utilization of plant genetic resources. Knowledge of genetic diversity and relationships among a set of germplasm and the potential merit of the genetic diversity would be beneficial in all phases of improvement, optimizing hybridization and selection procedures, besides understanding the cohorts in the event of sporadic, erratic and disjunct flowering in this bamboo species. The wide distribution and adaptability of *D. strictus* encompasses a great diversity which is expected to be reflected in its genetic constitution, hence identification and characterization of its genetic resources assumes great significance. The present work purports to study diversity in *D. strictus* germplasm using

biochemical traits.

MATERIALS AND METHODS

Twenty different accessions of *D. strictus* were selected from Dendrosetum at Forest Research Institute (FRI) Dehradun, harboring *D. strictus* clones collected from different eco-geographical zones of India in the year 2008 under the project **"**Bamboo improvement for rural and tribal communities, integrating new technologies*"* funded by National Bamboo Mission, New Delhi. Table 1.

Table 1

Serial. No	Accession code	Provenances	Latitude (°N)	Longitude (°E)	Altitude	
1	A-1	Biyasi	30°44′	78°27′	1352	**Uttrakhand, India**
2	A-5	Devprayag	30°15′	78°6′	830	
3	A-7	Mansadevi	26°92′	78°15′	444	
4	A-8	Haridwar	26°96′	78°16′	315	
5	A-10	Shyampur	26°74′	78°11′	310	
6	A-11	Sahaspur	26°73′	78°05′	311	
7	A-13	Kalsi	30°32′	78°03′	510	
8	A-16	Bhogpur	30°1′	77°28′	255	**Haryana**
9	A-17	Pinjore	30°79′	76°91′	550	
10	A-18	Thadugarh	30°73′	76°78′	225	
11	A-19	Seonthi	30°2′	74°23′	250	
12	A-20	Kurukshetra	29°6′	77°04′	222	
13	A-23	Hissar	29.15′	75.71′	210	
14	A-28	Ropar	30°96′	76°53′	262	**Punjab**
15	A-32	Kahanpur	26°46′	80°33′	228	
16	A-35	Hoshiarpur I	31°53′	75°92′	296	
17	A-36	Hoshiarpur II	31°52′	75°90′	294	
18	A-38	Dasuya	31°82′	75°66′	240	
19	A-40	Jhelwa	31°5′	75°6′	250	
20	A-88	Andhra Pradesh	17°36′	78°47′	536	**AP**

The following (in bold) biochemical constituents were studied and quantified in three seasons *viz.,* summer (March -May), rainy (June-August), winter (December - February) in three replicates (ramets) of each accession for two years.

Total Chlorophyll Content

The content of chlorophyll a and b was estimated using the method given by Holm (1954). The total chlorophyll content and chlorophyll *a / b* ratio were also derived.

100 mg fresh leaf material was homogenized with 1 ml of 80 % chilled acetone in mortar and pestle over ice pack and centrifuged at 2000 rpm for 10 minutes. The supernatant was elicited with 80% acetone and the absorbance was recorded in 644, 662 and 440.5 NM wavelengths spectrophotometrically in PC based UV-VIS spectrophotometer (Perkin Elmer Lambda 2S, German made) against 80% acetone. The formula as given by Holm was used to compute the chlorophyll content.

Total Soluble Protein

The total proteins from the leaves of 3 replicates expressed in mg/g fresh weight of leaves under all treatments was estimated by the method given by Lowry (1951). 0.1 m Tris-buffer (pH 7.5) in a cold, grinding medium with sodium

dodecyl sulfate (3%) and polyvinyl pyrrolidine (3%) followed by centrifugation at 2000 rpm for 10 minutes. 0.1 ml supernatant was taken and 0.9 ml extraction buffer was added to it along with 5 ml mixture of 2% Na_2Co_3 in 0.1 N NaOH and 0.5% $CuSO_4$ H_2O in 1% sodium potassium tartarate solution, in the ration 50:1. The tubes were kept for 30 min to allow color development. Absorbance was measured at 660nm against reagent blank in PC based UV-VIS spectrophotometer (Perkin Elmer Lambda 2S, German made). The standard curve was prepared with Bovine Serum Albumin (BSA).

Total Soluble Sugars

The estimation of total sugars was carried out as per the method given by Dubois *et al.*, (1956). 0.2 ml ethanol extract diluted with 0.8 ml-distilled water was treated with 1 ml of 5 % phenol and 5 ml concentrated sulphuric acid. The mixture was allowed to cool down to room temperature before finally recording the absorbance at 490 nm in UV/Vis spectrophotometer, (model Perkin Elmer, Lambda) against reagent blank in which the diluted extract was replaced by 1 ml distilled water. The standard curve was prepared by using D- Glucose concentrations. Total soluble sugar content was expressed as mg/g dry weight of a leaf.

Total Phenol Content

Total phenol content in the leaf samples was read from the reference curve prepared by using p-Cresol concentrations and expressed in mg/g dry weight of leaves. The ANOVA was carried out using Genstat version 3.2 as per the designed experiment i.e. randomized block design. The source of variation was accession, seasons and accession x season interaction. The F value, thus obtained was compared with the tabulated values at the 0.1 % level of significance and respective degrees of source and error.

The linear relationship between biochemical parameters were studied with the help of Minitab release 11.2 using Karl Pearson's simple correlation coefficients.

RESULTS

Total Biochemical Content

As evident from Table 2, Significant inter accession variation at the 5 % level was recorded in total soluble protein content with A35 from Hoshairpur recording (18.02 mg/g) of total soluble protein content followed by A13 (16.51 mg/g) from Kalsi. The total protein content among the accessions varied from 12.09 mg/g to 18.02 mg/g. The accession A35 (Hoshiarpur) registered the maximum amount of sugar content (25.24 mg/g) followed by accession 40(23.26 mg/gm) (Hoshairpur II).Similarly variation in the concentration of Chlorophyll pigment content across the accession is evident with maximum 2.55 mg/g observed in Hoshairpur A35 accession, however highest chlorophyll b pigment is found in A5.The secondary metabolites such as phenolics exhibited significant variation with accession A32 Kahanpur (Punjab) recording the maximum yield followed by A40 from Jhelwa (Punjab) .The range varied from A 32 (1.49 mg/g) to A23 (0.371 mg/g). The mean values for various biochemical components are given in Table (2).

Table 2: Biochemical Composition in a Score Selected Accessions of D.Strictus

Accessions	Total Chlorophylls (Mg/G)	Chlorophyll "A"(Mg/G)	Chlorophyll "B"(Mg/G)	Chlorophyll A/B	Total Protein (Mg/G)	Total Sugars (Mg/G)	Total Phenol (Mg/G)
A1	2.141	1.271	0.870	1.76	13.88	18.49	0.204
Table 2: Contd.,							

A5	2.309	1.420	**0.889**	1.83	12.58	21.01	0.14
A7	2.304	1.508	0.832	1.85	12.45	19.22	0.24
A8	2.229	1.614	0.614	3.60	14.98	21.48	0.11
A10	2.041	1.344	0.697	1.97	12.95	**15.58**	0.24
A11	1.343	0.869	0.474	2.13	15.2	19.07	0.22
A13	2.51	1.684	0.826	2.53	16.51	22.25	0.21
A16	1.579	1.067	0.512	2.39	15.43	19.68	0.10
A17	2.011	1.446	0.566	2.68	14.22	18.54	0.25
A18	1.857	1.392	0.464	3.47	15.92	19.63	**0.02**
A19	**1.013**	**0.668**	**0.346**	2.10	12.35	18.4	0.22
A20	1.543	0.969	0.574	2.06	15.01	17.19	0.09
A23	1.723	1.214	0.509	3.00	14.1	21.03	0.07
A28	1.913	1.144	0.780	**1.73**	15.03	16.1	0.24
A32	1.692	1.129	0.563	2.64	15.46	21.32	**0.30**
A35	**2.551**	**1.742**	0.809	2.44	**18.02**	**25.24**	0..12
A36	1.84	1.148	0.692	2.09	15.12	18.44	0.26
A38	1.971	1.447	0.524	3.74	15.82	18.89	0.25
A40	1.956	1.392	0.563	**3.77**	**12.09**	23.26	0.264
A88	1.756	1.109	0.647	1.72	13.27	21.77	0.155
Mean	**1.9**	**1.28**	**0.638**	**2.5**	**14.5**	**19.8**	**0.20**
Significance	***	***	***	**NS**	***	***	***
Critical Difference	**0.3445**	**0.333**	**0.238**	**1.67**	**1.786**	**3.511**	**0.034**

*** - Significance at 0. 1%, ** - Significance at 1%. * Significance at 5%, NS – Non Significant.

Significant interaction Table 3 between season and accession is reflected in total soluble sugar and protein content. The best interaction was observed in accession A18 (21.27 mg/g) from Thadugarh yielding maximum amount of total protein in rainy season *viz.*, July –August. The month viz., Dec-January found a consequential downtrend in translational concentrates. Similarly the photosynthates viz., sugar (28.57mg/gm), chlorophyll (3.153 mg/gm) also show best interaction in the month of July-August in accession A35. Phenolics, however exhibited best interaction in summer (may-june) in A32 (0.39mg/gm). Table 3

Table 3

	Protein			**Sugars**			**Cholrophyll**			**Phenols**		
	Summer	**Rainy**	**Winter**	**Summer**	**Rainy**	**Winter**	**Summer**	**Rainy**	**Winter**	**Summer**	**Rainy**	**Winter**
Maximum	20.84 (A35)	21.27 (A18)	14.75 (A35)	26.71 (A35)	28.57 (A35)	20.44 (A35)	2.893 (A35)	3.153 (A35)	1.607 (A35)	0.39 (A32)	0.31 (A40)	0.22 (A32)
Mean	15.08	18.04	10.44	20.95	24.95	13.59	2.063	2.713	0.972	0.29	0.199	0.10
CD (SEAS)	3.406	2.897	3.167	7.462	3.364	6.97	0.777	0.6341	0.327	0.065	0.064	0.05
Significance	***	**	**	*	***	NS	***	***	***	***	***	***
CD (S*A)	3.094			-			1.030			0.059		
Significance	**			NS			NS			***		

SEAS :seasons, S*A :Season x accession, CD: Critical Difference.

Maximum correlation is observed between total soluble sugars , proteins and cholophyll (0.54 to 0.697) whereas no perceptible correlation is found between total phenols and other biochemical parameters (less than 35%) Table 4.

Table 4: Correlation between Biochemical Parameters

Parameters	Total Phenol	Total Sugars	Total Protein	Total Chlorophyll
Total Phenol	1.000			
Total Sugars	0.267	1.000		
Total Protein	0.333	0.543*	1.000	
Total Chlorophyll	0.342	0.651*	0.697**	1.000

DISCUSSIONS

There are practical implications of the need to understand the variables existing within the species or a population of diverse geographical locations arising as a result of isolation or adaptation to different environmental conditions. The relative value of individual populations of a species is very difficult to judge in natural stands; therefore they were sampled and subjected to variation study in the laboratory as well as the field.

Spikes in chlorophyll content in the leaves of D. *strictus* in rainy season from July to August could be attributed to humid air temperature, possibly ideal for its chlorophyll biosynthesis. Kopsell and Kopsell (2003) also reported the chlorophyll biosynthesis to changing air temperature as highly species specific. The increased chlorophyll content in rainy season could also be partly due to the increased absorption of Mg^{+2} ion(s) the central atom amid four nitrogen atoms in a chlorophyll molecule, from soil to the root hairs. Positive correlation between rainfall and micronutrient availability has been reported by Barauh and Barauh (2000).The perceptible dip in chlorophyll pigments in low temperature winter season could be imputed to compromised pigment protein complexes eg thyllakoid membranes and subsequent photodynamic inactivation of chlorophyll due to minimal sunlight. Similar upsurges in chlorophyll content in rainy, summer and winter were reported by Senser *et al*., (1975) and Lewandowski and Jarvis (1977) for Sitka spruce. In a number of conifers, chlorophyll content shows maximum in summer and minimum in winter (Bourdeaeu, 1959 and Senser *et al*., (1975). Maximum chlorophyll content in rainy season possibly harvests more energy in the form of solar radiations converting more carbondioxide into photosynthates, thus more sugar content is obvious during the season. Also elongation and proliferation of bamboo culms takes place mainly during the rainy season, as humidity is the governing factor for growth in bamboo (Kadambi, 1949). The highest mitotic rate has to be commensurate by producing more sugars and other primary metabolites of physiological importance. The high soluble sugar content in *D. strictus* leaves in the rainy season are also associated with high catalytic activities of soluble invertases (Morris and Arthur,1985,Quick and Shaffer 1986, Ugalla *et al.*,2001) which convert sucrose into fructose and glucose and are later uploaded into expanding bamboo culm. The decrease in soluble sugars in winter can also be seen as a sort of adaptive strategy to enhance survivorship from seasonal pests, thus a positive correlation observed between chlorophyll content, sugar content and total soluble protein content is striking consonance with reports on other grass species by researchers. A similar high protein content in the rainy season was also found in dwarf bamboo *Sassa nipponica* (Yo Koyama *et al.,* 1988). Precipitation or the amount of rainfall in a particular geographical location spells a profound impact on the vegetation of the area. In China, productivity of bamboo dramatically increases with annual rainfall (Qui *et al*., 1992). Yoshito *et al*., (2000) who evaluated the seasonal variation in dry matter production and crude protein content in Nezasa type grassland found that crude protein content of Nezasa dwarf bamboo (*Pleioblastus Variegatus* Makino) was 20% more in rainy season compared to summer. Afolayan *et al*., (1978) also observed seasonal variation in protein content in tropical savanna grasses with the maximum protein content in the beginning of the growing season and declining towards the end. Overall, it was observed that genetic potentialities of accessions from Punjab were superior and expressed high levels of biosynthates and also displayed best interactions in rainy seasons, However, the best interaction of the season and accession on total protein content was observed in A18 from Thadugarh, Haryana (21.27 mg/g) in rainy season underscoring the favorable interplay of genes and environment. Significant variation was found with respect to phenol content the accession A32 Kahanpur (Punjab) registered the maximum yield followed by A40 from Jhelwa. Dry samples of leaf extracts registering maximum phenols in summer, could be imputed to stress conditions during high temperature conditions stimulating the synthesis of secondary metabolites such as flavonoids and phenols to combat environmentally

induced stress on its metabolic machinery.

CONCLUSIONS

Dendrocalamus strictus sps is characterized by a very erratic flowering cycle and thus a need to exploit artificial breeding programs like hybridization following screening of appropriate and desirable genotypes is warranted. This would not only reduce time in plantation trials, but the concomitant increase in anticipated outcomes. The wide range of diversity observed among the accessions could, thus, be employed to affect crosses and model varieties in a desired genetic architecture. The economic traits - culm growth ,number of clumps, internode diameter and culm biochemistry- are attributable to higher amounts of photosynthates and thus superior genotypes like A35 and A18 could be used as parent plant materials to acquire a desired heterotic response in future bamboo breeding programs .

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